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IN VIVO REPRODUCTIVE AND MUTAGENICITY TESTS

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16. ABSTRACT A review of current <u>in vivo</u> testing systems and procedures used for assessing reproductive and heritable genetic hazards of chemicals is presented. A comparison is made between the similarities and differences in protocols for reproductive effects among the different regulatory agencies in the United States and countries belonging to the Organization for Economic Cooperation and Development. Uses of <u>in vivo</u> mammalian mutagenicity data for assessing potential human heritable genetic diseases risk are also discussed and examples of how various <u>in vivo</u> mutagenicity test systems can be used to estimate risk are presented along with explanations of the various assumptions employed.		
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IN VIVO REPRODUCTIVE TOXICOLOGY

INTRODUCTION

Several standard protocols have been issued by various regulatory agencies as in vivo methods for use in determining the potential reproductive hazards of drugs and chemicals. All of the regulatory agencies have proposed in vivo methods for determining the teratogenic potential of a test material. Protocols for evaluation of other reproductive hazards are not consistently available.

All issued in vivo procedures are based on the 1966 Food and Drug Administration (FDA) Guidelines (1). The methods include those procedures proposed by the United States Environmental Protection Agency (EPA) (2, 3); Japan (4); Great Britain (5); Canada (6); the Organization for Economic Cooperation and Development (OECD), an international organization of 23 countries (7); and the United States Interagency Regulatory Liaison Group (IRLG) (8), an organization of six Federal agencies (Consumer Product Safety Commission, the Environmental Protection Agency, the Food and Drug Administration, the Occupational Safety and Health Administration, and the Food Safety and Quality Service of the Department of Agriculture).

These in vivo methods have been previously well described (9, 10, 11). The procedures are designed to evaluate critical periods for susceptibility to reproductive toxicity (12). Each set has minor variations in terms of the number of required tests and in the manner in which these tests are to be conducted. These requirements are summarized in Table 1. This table was developed on the basis of interpretation of the various guidelines and information available in other sources (13).

Reproductive hazard evaluation considers three populations at risk: the

TABLE 1. TESTING REQUIREMENTS*

	SEGMENT I	SEGMENT II	SEGMENT III	REPRODUCTION (2 OR 3 GENERATIONS)
FDA (1966)	M, F 1 SPECIES	F - 1 RODENT (20) F - 1 NON-RODENT (10)	F - 1 RODENT	F - 1 RODENT
EPA (PROPOSED FIFRA; 1978 TSCA; 1979)	--	F - 2 SPECIES (RODENT - 20; NON-RODENT - 12)	--	--
JAPAN (1975)	M, F 1 SPECIES	F - 1 RODENT (30) BEHAVIOR	F - 1 RODENT BEHAVIOR	F - 1 RODENT BEHAVIOR
GREAT BRITAIN (1974)	M, F 1 SPECIES SKELETAL, VISCERAL, AND BEHAVIORAL	F - 1 RODENT (24) F - 1 NON-RODENT (12) BEHAVIOR	F - 1 RODENT	F - RODENT BEHAVIOR "POSSIBLE"
CANADA (1973)	--	F - 1 RODENT (20) F - 1 NON-RODENT (10)	--	--
OECD (1979)	--	F - 1 RODENT (20) F - 1 NON-RODENT (12)	--	--
IRLG (1981)	--	F - 1 RODENT (20) F - 1 NON-RODENT (12)	--	--

*M = Male, F = Female, () = Number of animals to be evaluated

male, the female, and the conceptus. The standard FDA protocols evaluate these three populations. It should be noted that although these "state of the art" tests are limited in their potential to predict all possible effects, they are capable of demonstrating potential risks and indicating areas which require further evaluation.

The FDA studies are commonly referred to as Segment I, II, and III evaluations. For food additives a two or three generation reproduction study is also performed. Because all regulatory agencies used the FDA protocols as the basis for their particular evaluations, these protocols will be described along with specific differences required by other regulatory agencies.

PROTOCOL DESCRIPTIONS

Segment I Evaluations

FDA--

The purpose of the Segment I evaluation is to evaluate the potential of a test agent to alter fertility and general reproductive performance. Various tests are performed during the conduct of this study to assess the toxic effects of a test agent on gonadal function and mating behavior in both male and female animals; on conception rates; on the early and late stages of gestation, parturition, and lactation; and finally, on development of the offspring. This study is generally used as a basis for determining the specifics of the Segment II and III studies to be performed in the same species.

Because male reproductive capacity is evaluated only in the Segment I study, it is important that an adequate number of male animals be tested. A minimum of 20 male and 20 female rodents should be evaluated in a control group and in each of three dosage groups. In order to determine whether an

observed toxic effect is restricted to one sex, or whether treatment of both sexes potentiates the reproductive hazard, an additional group of 10 male and 10 female animals is frequently added to both the control and high dosage groups (14). These additional animals are cross-mated so that information is gained as to the effect of agent treatment of only one sex.

The rat is the most frequently used test species. Both male and female animals should be sexually mature when assigned to study. Male rats are administered the test agent for a minimum of 60 days prior to and through a cohabitation period. Estrous cycling may be evaluated in female rats prior to initiation of treatment with the test agent. The test agent is administered to female rats for 14 days prior to cohabitation. Estrous cycling should be evaluated for agent-related changes during this precohabitation period. Female rats are treated with the test agent until they are killed and examined following Caesarean section on day 13 of presumed gestation, following observation of non-pregnancy (greater than 25 days of presumed gestation) or following natural delivery and weaning of pups. The supplemental control and high dosage group rats are cross-mated, and all of these female rats are permitted to naturally deliver and nurse pups.

In initial matings, one male and one female rat should be placed in cohabitation. Mating performance of male and female rats may be evaluated on the basis of observation of either spermatozoa in a daily smear of vaginal contents and/or a copulation plug. Day 0 of gestation should be defined as the day on which insemination is confirmed.

One-half of the female rats in each dosage group, selected either randomly or on an odd-even basis, are sacrificed on day 13 of presumed gestation and examined for number and distribution of corpora lutea, implantations, and live and resorbing embryos. Any condition in either the dam or sire which may have

contributed to reduced fertility should be noted.

The remaining one-half of the female rats in each dosage group are permitted to naturally deliver and nurse pups. These female rats should be evaluated for the duration of gestation, calculated as the number of days from day 0 of presumed gestation to the day the entire litter is delivered. Delivered litters should be examined for number, viability, gender, body weight, and gross morphological variations in pups. Survival of the dam and pups and maternal and pup body weights should be recorded at birth (day 1) and on days 4, 7, 14, and 21 postparturition. Random culling, preferably to four male and four female pups per litter, is frequently done on day 4 postparturition, in order to standardize litter sizes. Stillborn or moribund pups may be preserved for additional studies.

Variations--

Japan--The protocol required by Japanese regulatory agencies is essentially the same as that proposed by the FDA. The only significant difference from the FDA protocol is that maternal treatment is stopped at initiation of major embryo organogenesis, rather than continuing throughout maternal gestation and lactation. Thus, treatment in the rat is discontinued on day 6, and in the mouse, on day 7 of gestation.

Great Britain--For submission to British regulatory agencies several alterations of the FDA protocol are required:

1. Dams are not killed and examined on day 13 of presumed gestation, but rather are examined on day 20 of presumed gestation. Caesarean-delivered day 20 fetuses are examined for viability and gross external malformations. Following the gross external examination, these fetuses are preserved for

possible subsequent examination for soft tissue and skeletal variations.

2. Rather than terminating naturally-delivered litters at weaning, selected pups are evaluated postnatally for general sensory (auditory, visual) and "behavioral" development and for fertility.

EPA, OECD, and IRLG--A Segment I evaluation is not currently required by these regulatory agencies.

Segment II Evaluations

FDA--

The purpose of the Segment II evaluation is to determine the teratogenic potential of the test agent. FDA Segment II evaluations are generally performed in rats and rabbits, as both a rodent and a non-rodent species are proposed for evaluation. The day spermatozoa, a vaginal plug (rats, mice), or insemination (rabbits) occurs is defined as day 0 of presumed gestation. The test agent is administered to animals during the period of major organogenesis. Caesarean sections are done one or two days before the presumed date of natural delivery. Currently accepted treatment periods used in the commonly evaluated test species are:

<u>TEST SPECIES</u>	<u>TREATMENT PERIOD (DAYS OF GESTATION)</u>	<u>CAESAREAN SECTION (DAY)</u>
<u>RODENT</u>		
RAT	6 - 15	20
MOUSE	6 - 15	18
HAMSTER	6 - 12	15
<u>NON-RODENT</u>		
RABBIT	6 - 18	29 or 30

The uterus is exposed and examined for the number and placement of implantation sites, early and late resorptions, and live and dead fetuses. The ovaries are removed and examined for the number of corpora lutea.

Weights of all fetuses are recorded, and each fetus is examined for gender and gross external variations. In studies of rodent species, one-third of the fetuses in each litter are examined for soft tissue malformations using such standardly accepted methods as Wilson's sectioning technique (15) or Staples' visceral examination technique (16). The remaining two-third's of the fetuses in each litter are stained with alizarin red-S (17) and subsequently examined for skeletal malformations.

In studies of non-rodent species, all fetuses are examined for both soft-tissue and skeletal malformations.

The FDA suggests using 20 rodents and 10 non-rodents per dosage group. Rodent studies are performed using a control and three dosage levels. In non-rodent studies, only a control and two dosage levels are required, although generally three are used. All regulatory agencies except the FDA require three.

Recommended Practices--

When the generally-accepted treatment periods were cited in 1966, implantation times in the various species were not accurately determined. Now that the times of histologic implantation are better known, it is considered appropriate to use treatment times such that exposure to the test agent does not interfere with implantation. If treatment is not initiated during nidation, but delayed until after histologic implantation has occurred, the compound can be tested for its teratogenic potential without the confounding factor of its possible ability to interfere with implantation. Using the

landmarks for determining the period of major organogenesis, histologic implantation to palatal closure (18), the appropriate treatment times to be used in the various species are:

<u>TEST SPECIES</u>	<u>TREATMENT PERIOD (DAYS OF GESTATION)</u>
<u>RODENT</u>	
RAT	7 - 15
MOUSE	7 - 15
HAMSTER	6 - 12
<u>NON-RODENT</u>	
RABBIT	8 - 19

Since it is not possible to evaluate teratogenic potential of a test agent in non-pregnant animals, the minimum number of female animals cited for evaluation should be pregnant. Thus, it is appropriate to assign more than 20 animals to each dosage group, as a pregnancy rate of greater than 80% generally cannot be guaranteed.

EPA--The EPA proposed guidelines differ from those required by the FDA protocols in several ways.

1. Two species are evaluated, but a rodent and non-rodent species is not required.
2. Soft tissue evaluations are performed in one-half of the fetuses in each litter, and skeletal evaluations are performed in the other one-half of the fetuses in each litter.
3. Recording and evaluation of placental, uterine, and maternal carcass weights are suggested, although the inherent variability present in these parameters makes their significance questionable.

4. The maternal treatment period is extended to the day prior to caesarean-sectioning, rather than including only the period of major organogenesis. The hypothesis for this prolonged treatment period is that treatment during both the embryonal and fetal periods was considered to increase the probability of demonstrating an agent's teratogenic potential. The EPA protocols require the following treatment times:

<u>TEST SPECIES</u>	<u>TREATMENT PERIOD (DAYS OF GESTATION)</u>	<u>CAESAREAN-SECTION (DAYS)</u>
RAT	6 - 19	20
MOUSE	6 - 17	18
HAMSTER	6 - 14	15
RABBIT	6 - 28 or 29	29 or 30

Although currently proposed, this extended treatment period does not seem appropriate for the following reasons:

- a. The effects of the test agent may be altered by prolonged treatment resulting in enzyme induction or suppression.
- b. It may be required to evaluate lower maximal dosages because of increased maternal toxic response.
- c. The literature indicates that fetal insult is associated with functional, rather than gross morphological changes (16). Functional changes may be detected in postnatal evaluations, but these would not be apparent in caesarean-derived fetuses evaluated for alteration of organogenesis or ossification. Thus, extending the treatment period into the time of fetal growth, beyond the period when major organogenesis occurs, will most probably result in functional changes which will not be detected in the required gross morphological evaluations.

- d. The number of fetal deaths will probably be increased as a result of the prolonged treatment period, with a resultant loss of fetal material available for examination.
- e. The cost of performing the study is increased by extending the treatment time, without a significant increase in usable information.

Japan--Differences from the FDA protocol required for submission to Japanese regulatory agencies are:

- 1. Thirty rodents are evaluated in each dosage group.
- 2. Rats are treated on days 7 through 17 of gestation.
- 3. Twenty of the 30 dams undergo caesarean section on day 20 of gestation; the remaining 10 dams are permitted to deliver litters naturally.
- 4. Postnatal evaluation of maturation and physical development is required as well as some measurement of locomotion, learning, sensory function, or emotionality. The fertility of the F1 generation is evaluated as well as the gross anatomy of the F2 generation fetuses.

Great Britain--Differences from the FDA protocols required for submission to British regulatory agencies are:

- 1. Evaluation of 24 rodents and 12 non-rodents per dosage group is recommended.
- 2. In certain cases (undefined), postnatal "behavioral" evaluations are required.

OECD AND IRLG--These guidelines currently differ from the FDA guidelines in that 20 pregnant rodent and 12 pregnant non-rodent animals are recommended for use in each dosage group. Although recording of carcass weight is recommended, this parameter, as previously discussed, is not considered appropriate for evaluation.

Segment III Evaluations

FDA---

The purpose of the peri-and post-natal evaluation is to assess the effects of a test agent on the development of the fetus during the last one-third of gestation, as well as any adverse effects of the agent on delivery, lactation, and postparturition pup development. The evaluation is generally performed in rats. Dams are inseminated by untreated male breeders. The test agent is administered to dams beginning on day 15 of presumed gestation and continued through delivery and a minimum of 21 days postparturition. The duration of gestation, delivery, and any delivery complications observed at parturition are evaluated. The number and viability of naturally-delivered litters are evaluated during the postparturition period. Cross-fostering of a group of control and high dosage group litters is frequently performed if poor pup survival was observed in the Segment I evaluation of the test agent.

Japan--

The Japanese regulatory requirements differ from those of the FDA as follows:

1. Postnatal development and "behavioral" evaluations of F1 generation pups are performed
2. Treatment of dams is initiated on day 17 of presumed gestation.

EPA, IRLG, and OECD--

These regulatory agencies do not currently require a Segment III evaluation.

Reproduction (2 or 3 Generations) Evaluations

FDA and EPA--

The studies are generally performed when food additives are evaluated (FDA) or as a third study for submission of agents regulated by the EPA. In EPA submissions, this test serves as a replacement for the FDA Segment I and Segment III evaluations.

The study is usually performed in rats. Generations are cited as Fo, F1, and F2. Treatment is initiated in the Fo generation immediately after weaning and then continued through mating, delivery, and nursing of the F1 generation pups. A second mating of the Fo parental generation may be evaluated but is no longer generally performed. Individual treatment of the F1 generation pups is begun at weaning, which usually occurs when they are 21 days of age. Treatment of these rats continues through mating and delivery of F2 generation pups.

The FDA and EPA protocols have two primary differences.

1. The EPA requires evaluation of two generations of rats while the FDA requires evaluation of three generations of rats.
2. The FDA protocol requires target organ evaluation, while the EPA protocol requires histopathology of all organ systems.

Japan and Great Britain--

For submission to Japanese regulatory agencies, postnatal "behavioral" assessment is required. The British also have "behavior" as a possible

additional requirement. Submission of food and water consumption data, ophthalmologic evaluations, and organ weight data are frequently requested.

IRLG and OECD--

Reproduction evaluation guidelines are proposed but are not yet issued by these groups.

DISCUSSION OF PROTOCOLS WITH RECOMMENDATIONS

Most of the protocols require that the evaluations be performed using a no effect level and maximum tolerated dosage. Because different endpoints are evaluated, these ideal dosage requirements cannot always be met. For instance, a few minor maternal effects may be observed at the lowest dosage evaluated, or the highest dosage that can be administered may produce no maternal effect. The real question to be addressed in a study is whether there is a unique hazard to the population examined, the sire, the dam, or the conceptus, not whether any hazard exists. Thus, in a teratology study, it is not necessary to demonstrate that no hazard exists, but rather than no unique hazard to the conceptus exists.

The currently proposed evaluations frequently confuse endpoints in an attempt to evaluate multiple endpoints in single studies. Since regulation must be based on a particular endpoint, multiple effects can have great significance. For example, as currently proposed, the TSCA and FIFRA two generation reproduction studies are fair evaluations of reproductive hazard and inadequate tests of carcinogenic potential. However, these EPA protocols attempt to evaluate both endpoints. The studies are based on a format originally used for nutritional studies, in which it was considered appropriate to use several generations of animals to evaluate the effects of

prolonged undernutrition. The endpoint evaluated was reduced litter size. The FDA (1970) proposed that the accumulation of toxins could be appropriately evaluated in this same model, because accumulation of toxins was considered to be the reverse of loss of nutrients (19). The processes of nutritional loss and toxin accumulation are not necessary mirror images. The EPA protocols also added extensive necropsy and histopathology procedures, thus changing the reproduction study into a short-term chronic toxicity/carcinogenicity evaluation. These considerable increases in evaluation requirements were proposed even though long-term carcinogenicity evaluations in two species were already part of the submission package.

The Segment II teratology evaluation treatment period extension, proposed by FIFRA and TSCA, and the collection of additional measurements in caesarean-derived pups similarly confound interpretation of teratogenic effects. Abortions are increased in all study groups, metabolic changes may be produced in the dams, it may be necessary to evaluate lower dosages, and uninterpretable data may be collected.

Each of the currently used screens has some major obvious design deficiencies, as discussed. Several are quite insensitive (20). Generally, the new protocol designs incorporated the old defects and added new measurements, such as "behavioral" evaluations (21, 22, 23). These new measurements may obscure even those few endpoints which can be validated: life, death, and gross anatomical malformation.

The in vivo evaluations currently used provide valid, reproducible information in test species at the levels of life, death, and gross change. Standardization and validation of endpoints more subtle than life and death are not available at present.

IN VIVO MUTAGENICITY TESTS

INTRODUCTION

Historically, short-term mutagenicity test systems have been used primarily to provide evidence in support of the carcinogenic potential of chemical agents. If positive results are obtained, then more elaborate long-term in vivo chronic bioassays for carcinogenicity may be called for in order to assess more fully a chemical's carcinogenic potential. The principle underlying the use of mutagenicity tests as predictors of potential chemical carcinogens is based on the somatic mutation theory of cancer in which an agent induces mutations that structurally change DNA, causing certain cells to transform with eventual progression to malignancy. The empirical observations that a large proportion of chemicals are both carcinogenic in mammals and give positive responses in short-term mutagenicity tests support the use of these tests as predictors of carcinogens.

It is estimated that at least 10.7% of all live-born humans will sometime be seriously handicapped by a genetic disorder (24). These structural and functional health impairments adversely affect the individual's societal function and economic survival. An agent with mutagenic properties may therefore not only cause cancer in the exposed individuals but may also increase the incidence of genetic disease in future generations. There is ample evidence that this occurs in experimental mammals. Thus far, no direct data are available to demonstrate that environmental agents are responsible for the occurrence of genetic diseases in humans. This is not surprising, since human populations are genetically heterogeneous and mobile, and some of the mutagenic effects would not be manifested until several reproductive generations after exposure. However, some are expected to occur in the

immediate offspring. Although the number of different genetic diseases is large, individual frequencies are low (only a few being more common than 1 per 1000). Furthermore, humans bear relatively small numbers of offspring, and the retrospective quantification of exposure to a particular agent is extremely difficult because large sample sizes are required to detect genetic disease.

Many chemical agents used in foods, drugs, and consumer products, and also for industrial processes have been shown to be mutagenic in mammalian and submammalian test systems, and thus, may have the potential to increase genetic damage in future human populations. Because of these findings, mutagenicity, as it relates to heritable effects, is becoming recognized as a distinct toxicological endpoint of public concern. Federal statutes, such as the Toxic Substances Control Act, provide the authority to regulate chemicals that are potential human mutagens capable of causing heritable genetic diseases. The U.S. Environmental Protection Agency has recently proposed Guidelines for Mutagenicity Risk Assessments (25) that suggest test systems which may be used to assess mutagenic risks and provide a means for analyzing mutagenicity data obtained under the Agency's various statutes.

Genetic alterations of concern can be functionally classified as: (a) chromosomal aberrations, which involve either structural changes in chromosomes or numerical changes (i.e., gains or losses of whole chromosomes or sets of chromosomes), and (b) gene alterations, which involve changes in the base sequences of DNA (e.g, base substitutions, frameshifts, and minor deletions). Many screening tests have been developed to score for either of these genetic endpoints.

The results obtained from screening tests can only be used to determine the intrinsic mutagenicity of environmental agents. Only mutations occurring

in whole animal tests involving sexual reproduction can be shown to be transmissible to future generations, and mammalian tests are therefore desirable for use in heritable genetic disease assessments. In the remainder of this section, consideration will be given to whole animal test systems that have been or are being developed, and more specifically, to those that measure recoverable heritable damage that is a consequence of gene alterations or chromosomal aberrations induced in germ cells. An example of how each of these tests may be used in assessing risk to humans is presented and is accompanied by a discussion of some of the assumptions necessary for making the assessments.

GENE MUTATION TEST SYSTEMS

Mouse Specific Locus Test Using Visible Markers

In 1951, W.L. Russell (26) developed a method for scoring heritable mutations induced at multiple specific loci of the mouse. Seven loci were chosen whose phenotypes can be visibly scored in the first generation offspring of treated parents. These loci control coat pigmentation, eye color, and external ear morphology (27, 28, 29, 30).

The method involves treating wild-type (101 x C3H) F_1 males and mating them with Oak Ridge test stock females which are homozygous for a recessive marker at each of the seven loci. The first-generation offspring have the same phenotype as the wild-type parent, unless a mutation has occurred at one of the marked loci in the treated parent. The interval between treatment and conception indicates in which germ-cell stage a mutation occurred (31).

The specific locus test has been in existence for 30 years, and extensive control data are therefore available. Because these are consistent for

several laboratories, they could be combined to yield a historical control over 800,000 (31). The spontaneous mutation rate was determined to be 7.7×10^{-6} mutations per locus for males (31) and 6×10^{-6} for females (32). In view of this large historical data base, it may not be necessary in all cases to use a contemporary control group when testing the mutagenicity of chemical agents. The large control sample and low rate also make the test sensitive. Thus, potent mutagens can often be detected with samples of a few thousand and many can be classified as nonmutagens as described by genetic toxicology criteria with a sample size of 12,000 (31).

Only 25 chemical agents have been tested in the mouse specific locus test using visible markers (31) as described by Russell (26, 27), even though it is the most proven multiple loci method available. Thus far, nearly all suspected chemical mutagens tested have been injected intraperitoneally in wild-type males at one concentration which represents the highest tolerable dose that does not elicit a severe toxic response. Only a few laboratories in Europe and America have the animal facilities sufficient for breeding large numbers of animals. However, no special expertise beyond a familiarity with mouse phenotypes is necessary for scoring heritable mutations, and 375 animals can be scored per hour per person.

For risk assessment purposes, the visible marker method can be used to detect agents capable of causing heritable mutations to determine their potency and to determine which stage of spermatogenesis is most sensitive to mutagenesis. Because it is impractical to produce the large numbers of animals that would be required to yield statistically significant results at low levels of effect (namely, induced rate $< 4 \times$ control), high-to-low dose extrapolations, similar to those used for cancer data (33, 34) for many chemicals, must often be made in order to estimate human mutagenic risk for

ambient exposures to chemical mutagens. This, of course, raises several questions. Is the slope of the dose-response curve at high levels of exposure the same as at low levels? Might there be repair at low doses? At high doses can toxic effects and changes in pharmacokinetics and metabolic pathways come into play which will influence the mutational response?

After making some reasonable assumptions, the human risk of heritable genetic disease from exposure to mutagenic agents can be estimated from the mouse specific locus data by an indirect method (24). From the animal studies the number of mutations per gene can be determined by scoring for the number of mutations at the seven visible loci and obtaining an average mutation frequency per locus after correcting for the spontaneous mutation frequencies. This value is taken as the increased incidence of mutations per locus caused by exposure to the environmental agent in mice. The ratio of the mutation frequency per locus (as a result of exposure to a mutagenic agent) to the spontaneous mutation frequency per locus (based on the mouse data) can then be determined. In order to estimate the increase in heritable disease incidence, this ratio is multiplied by the spontaneous heritable disease incidences in humans caused by gene mutations. This would provide an estimate of the increased incidence of heritable genetic diseases, if the exposure received by humans is comparable to that received by experimental mice. The relationship can be expressed as

$$H_e = \frac{F_c}{F_s} (S + I)$$

where

H_e = the increased heritable genetic disease incidence caused by gene mutations at a given exposure of the chemical

F_c = the mutation frequency per locus/generation in mice caused by the environmental agent (corrected for spontaneous mutation frequency)

F_s = the spontaneous mutation frequency per locus/generation
(7.7×10^{-6}) (31)

S = 2000 disorders per million live births. This value represents the existing dominant and x-linked mutations expected in the first generation. The mutational component of genetic disease was assumed to be 1 (35).

I = 450 disorders per million live births. This value represents the estimated number of genetic disorders expected in the first generation caused by irregularly inherited mutations. The mutational component of genetic disease was assumed to be 0.05 (35).

The actual exposure humans receive may be quite different from that of mice, and appropriate correction factors, dependent on the route of exposure, are needed. For further details, see the paper by Mantel and Schneiderman (36).

Other major assumptions made when using the above procedure include:

1. The visible loci scored in mice are representative of all genetic loci found in mice and humans.
2. The animal studies are conducted at high dose levels, and, provided there was no excessive spermatogonia killing, linearity of the dose-response curve to zero dose is assumed in order to estimate the heritable genetic disease incidences at lower human exposure levels.
3. In the above formula it is assumed that both males and females have the same susceptibility to chemical mutagens. For radiation, however, the BEIR III Committee felt that females may be only 40% as sensitive as males (24). Unfortunately, not enough is known about differences in chemical mutagenic susceptibility between sexes.

A more elaborate account of the assumptions used for the indirect method of estimating heritable genetic disease risks can be found in the reports of the United Nations Scientific Committee (35) and the Committee on the Biological Effects of Ionizing Radiation (BEIR III) (24).

Mouse Specific Locus Test Using Electrophoretic Markers

Methods to detect ionizing radiation-induced heritable mutations in mice using electrophoretic techniques were first developed by Valcovic and Malling

in 1973 (37). More recently, the chemical agents, triethylenemelamine (38), procarbazine (39, 40), and ethylnitrosourea (41) have been reported to induce gene mutations which are expressed as electrophoretic differences in cellular proteins. The test involves exposing parental males to an environmental agent, mating them to untreated females, and then comparing the electrophoretic patterns in starch gels of marker proteins isolated from red cell lysates and kidney homogenates in the F₁ generation. The test uses common inbred strains (C57BL/6 and DBA/2) which differ genetically at ten autosomal protein marker loci. These differences enable the detection of mutations in the treated parent by measuring losses of enzymatic activity and alteration of electrophoretic mobility. An important feature of this test is that it is possible to determine in which parent any mutation occurred. Although impractical, it would be possible to treat both parents in an experiment.

Ten additional loci have been identified in which only electrophoretic mobility changes can be readily detected. The parental source of the mutation cannot be determined in these cases. The first ten loci contain the following proteins: soluble maleic enzyme, esterase 1, esterase 3, isocitrate dehydrogenase, beta chain of hemoglobin, peptidase 3, hexose 6-phosphate dehydrogenase, and phosphoglucomutase 1 and 3. The second group of ten loci contains two esterases, two lactate dehydrogenases, three peptidases, a phosphoglucomutase, a 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase. Since mutations can be scored from more loci in the biochemical specific locus test than the morphological locus test, the former may provide a more sensitive means to assess mutations. However, more studies examining chemically-induced mutation frequencies between both systems are necessary.

In order to calculate the average induced gene mutation frequency, the total number of electrophoretic changes are scored and divided by 20 times the number of F₁ offspring examined (42). When such data are available, the human risk of heritable genetic disease from exposure to chemical agents can be estimated using the same indirect method previously described for the visible specific locus marker test. The major assumptions made for the visible marker test also apply to the test using electrophoretic markers.

A comparison has been made between the morphological (visible markers) and biochemical (electrophoretic markers) specific locus tests using one chemical, ethylnitrosourea (ENU). Russell et al. (43), using visible markers, treated male mice with a single interperitoneal injection of 250 mg/kg ENU and reported an induced gene mutation incidence of 53×10^{-5} per loci. Johnson and Lewis (42), using electrophoretic loci, treated male mice with the same dose of ENU and found an induced gene mutation incidence of 42×10^{-5} . These results show agreement between the two test systems.

Although the biochemical marker method involves surgical removal of kidney tissue, extraction of peripheral blood, sample preparation, and electrophoresis, it does, nevertheless, have several potential advantages. More loci can be added, and the biochemical consequences of the mutation can be studied. Furthermore, Neel et al. (44, 45) have undertaken a gallant effort to screen human populations in an attempt to detect electrophoretic mutations. Since many of the proteins being examined are similar to those in the mouse, future comparisons between the two species may provide a better understanding of the species variability.

Dominant Skeletal Mutation Test

In 1966, Ehling (46) observed that male mice exposed to X-irradiation at high levels produced a high frequency of dominant mutations that caused

skeletal malformations in the F_1 generation. More recently, Selby and Selby (47), in a much larger study incorporating breeding tests, found 37 dominant skeletal mutations in 2646 F_1 progeny of male parents exposed to 100R + 500R (24 hours between fractions) of acute gamma radiation. Only F_1 's derived from treated spermatogonia were involved in this study. For purposes of risk estimation and in the absence of a concurrent control, the induced mutation frequency was taken to be 1.4% per gamete. From Ehling's studies (46) the spontaneous mutation frequency in a large control sample was very low and was considered as a negligible correction in the above calculation. After correcting for the fractionation effect of radiation and the dose rate effect, the estimated induced mutation rate under protracted exposure was calculated to be 4×10^{-6} mutations per gamete per rem (24, 48).

This value was taken as the estimated frequency of induction for all detectable dominant mutations affecting the skeleton. However, in estimating risk the amount of clinically-important dominant damage affecting all body systems must be determined. Based on phenotypes of the 37 mutations, it was assumed that 1/2 (35) or between 1/4 and 3/4 (24) would be of clinical importance in humans. It was also assumed that the dominant mutations affecting the skeleton represent 1/10 (35) or 1/5 to 1/15 (24) of all dominant mutations. These factors are based largely on (a) a tabulation of monogenic disorders in humans by McKusick (49) which revealed that about 1/5 of clinically-debilitating dominant disorders involve the skeleton, and (b) upon adjustments for the ease of diagnosis of skeletal defects and pleiotropy. Multiplication of the frequency of 4×10^{-6} dominant skeletal mutations per rem by 1/2 and 10 as described in the UNSCEAR report (35) yields a risk estimate of 20 serious dominant disorders per million live born humans

following such paternal exposure. The relationship just described can be formulated as

$$H_e = (N) (5)$$

where N = the incidence of number of serious dominant skeletal defects in mice caused by exposure to a given agent corrected for spontaneous incidence.

5 = the factor to account for all dominant diseases.

This estimate of damage in humans relates to simple dominant disorders, those irregularly inherited disorders caused by dominance with incomplete penetrance and any gross chromosomal disorders that show dominant inheritance (24). The number of animals necessary to estimate risk is likely to be an order of magnitude less than the number of animals used in the morphological specific locus test, but more time is required to prepare and examine the skeleton for abnormalities. However, in the dominant skeletal mutation test, actual genetic damage resulting from mutations is directly measured, the types of abnormalities are easily recognized, and the abnormalities are easily associated with human skeletal defects.

Some of the major assumptions made in using this direct method for estimating the increased heritable genetic disease incidence are:

1. The induced mutation frequency in mice is approximately the same as the induced mutation frequency in humans.
2. If there is no long sterile period, the low-dose response can be estimated from the result at high doses by linear extrapolation.
3. The amount of proximate mutagens getting to the germ cells is equivalent in mouse and humans.
4. The clinically important damage induced in the skeleton represents a constant portion of total induced dominant damage (about 10%).

For ionizing radiation, the BEIR III Committee (24) reported that the increased incidences of serious dominant heritable disorders per million first generation live births after exposure of the entire population to 1 rem of radiation would be between 5 and 65, using the dominant skeletal mutation data. Values between 10 and 130 were obtained by the indirect method with the visible specific locus test in which the rates of elimination of mutants were assumed to be 5 generations for dominant disorders and 10 generations for irregularly inherited disorders (35). So far only one chemical, ethylnitrosourea (ENU), has been examined using this method (48) in which 150 mg/kg of ENU was shown to induce a high frequency of clinically important disorders.

Dominant Cataract Mutation Test

Another assay that detects heritable effects based on the scoring of dominant mutations has been described by Kratochvilova (50) and Kratochvilova and Ehling (51). In this assay, induced dominant cataract mutations have been scored in mice after exposure to gamma radiation or chemicals. The procedures used are similar to the dominant skeletal test system except that the mutations affecting the lens in the F_1 generation are assessed macroscopically with a slit lamp.

As tabulated by McKusick (49), about 3.1% (51) of the 583 well-established dominant heritable diseases in humans are associated with various forms of cataracts. Therefore, a factor of 32, instead of 5 for dominant skeletal defects, would be used in the equation on page 24 for estimating the increased human heritable genetic disease incidence.

Although cataract mutations are responsible for fewer dominant disorders in humans than are skeletal mutations, the lens disorders are more easily

scored. To date, no chemicals have been adequately tested for mutagenicity in the dominant cataract test.

Dosimetry Approach For Assessing Genetic Risk Caused By Gene Mutations

Major problems in extrapolating toxicological responses from one species to another are introduced by differences in pharmacokinetics and metabolic properties involved in activation and detoxification. For heritable gene mutations to occur, the critical target, DNA, has to be altered in the germinal tissue. Since DNA is essentially the same for all species, it appears that actual dose to the critical target molecule would be a more universal measurement than external exposure to the organism, and would be independent of metabolic and pharmacokinetic differences among species. Several investigators have shown that a linear relationship exists between binding of labelled mutagens (ethylmethanesulfonate and ethylene dibromide) to germinal DNA and induced mutation incidences in several nonmammalian test systems (52, 53, 54, 55). However, the correlation between exposure and mutation frequency was nonlinear. This method of measuring DNA dose through radioactively labelling the mutagen provides a sensitive measure to show a response at low enough levels where extrapolation to environmental levels have greater precision than the linear extrapolations used in the previous test systems described. For example, mice exposed to ethylene dibromide at 0.5 ppm for about 3 hours resulted in 4×10^{-8} alkylations per nucleotide in male germinal DNA (55, 56). Estimates of exposure of fumigant workers to ethylene dibromide are as high as 1.2 ppm (57). The experimental level is close to human exposure levels; however, a nongenetic effect and not an increase in mutation frequency is being determined in the whole animal.

Because of the ease and economic advantages of conducting nonmammalian gene mutation tests, a dosimetry approach is being considered for estimating mutagenic risk. This involves correlating induced gene mutation frequencies with alkylations to DNA in systems such as the sex-linked recessive lethal test in Drosophila melanogaster (55, 58) and correlating exposure (e.g., oral, inhalation) of a mutagen and alkylations to germinal DNA in whole mammals. The common entity measured in the whole mammal and in the gene mutation test is alkylation of DNA. With this bridge, a relationship can be made between induced gene mutation and exposure in the whole animal. The dosimetric method can then be used to estimate the increased incidence of heritable disease caused by human exposure to the environmental mutagen. Table 2 outlines the steps for using the dosimetry approach for risk estimation.

TABLE 2. OUTLINE OF THE PROCEDURE USED TO ESTIMATE HERITABLE GENETIC DISEASE RISK IN HUMANS FOR ETHYLENE DIBROMIDE

Estimate of total human exposure during the reproductive age.

Measurement of alkylations of germinal DNA in mice at this exposure

Relating the same multiplication of the number of doubling doses* to the incidence of heritable genetic disease in humans.

Calculation of the number of doubling doses* in the test system (Drosophila) caused by a comparable amount of DNA alkylation in mice.

*A doubling dose is a dose that will induce a mutation frequency equal to the spontaneous frequency.

There are several disadvantages to the dosimetry method. Mutations in a whole mammal are not being scored, and the mutations induced cannot directly be shown to be heritable. In using the dosimetry approach, one assumes that

if the chemical is mutagenic in a nonmammalian test system and alkylates DNA in mammalian germ cells, mutations are occurring in mammalian germ cells proportional to mutation incidences per DNA alkylation in the test systems. Only chemicals that cause mutations through binding to DNA can be used to measure alkylations. Complex mixtures in which the chemical structures aren't known, cannot be labelled, and therefore, cannot be tested. Other assumptions include:

1. The mutation frequency per alkylation in the test system is representative of both mice and humans.
2. Alkylation to DNA in all germ cell stages is the same.
3. The activation system in the gene mutation test (for those mutagens which need to be metabolized to be active) is the same as that in the whole mammal where DNA alkylation is measured.
4. Reciprocity is maintained in that a dose given over a short period results in the same total amount of DNA damage (alkylation) as the same dose given over a longer period (59, 60).

In spite of the need for additional assumptions, which are not necessary in mammalian tests, the dosimetry method has three distinct advantages. Many laboratories are capable of carrying out DNA labelling experiments as well as many of the in vitro gene mutation screening tests presently available. Chemical exposures can be much lower and more representative of actual human exposure, so that high dose to low dose extrapolations need not be made. The in vitro and nonmammalian gene mutation tests available are more powerful in scoring for gene mutations. The cost of conducting a whole animal labelling experiment and measuring gene mutations in nonmammalian test systems is much less than the whole animal mutation tests. However, the dosimetry approach needs to be validated by testing many chemicals and making comparisons with the other gene mutation test systems used for the assessments described above.

CHROMOSOMAL MUTATION TEST SYSTEMS

Dominant-Lethal Test

Dominant-lethal mutations are genetic changes in parental germ cells that cause death of the affected first-generation progeny. It is generally believed that induced dominant-lethal mutations are almost exclusively chromosome breakage events leading to deletions and exchanges (61, 62). The dominant-lethal test has been used extensively in studying genetic effects of ionizing radiation (63, 64) and in determining the mutagenic potential of environmental chemical pollutants (65, 66). The dominant-lethal effects induced by clastogenic agents are manifested in the death of conceptuses during the course of various stages of development. Animals (usually groups of 10 mice per dosage) are given either single doses, daily doses for five consecutive days, or daily doses for 8 weeks, with one level being the maximally-tolerated dose. The routes of administration selected should be the most relevant to human situations. Immediately following the last treatment, each male is mated with untreated virgin females. Males given a single dose and males given five consecutive daily doses are mated serially for at least 8 weeks to sample germ cells at all spermatogenic stages. Males given daily doses for 8 weeks, on the other hand, would have had their germ cells exposed to the chemical throughout the time spermatogenic cells are going through the maturation process. Consequently, these males are mated for only one week beginning immediately after the end of exposure. All mated females are killed for uterine analysis 12 to 15 days after observation of the vaginal plug. Mutagenicity of the test compound is decided by a combination of the following criteria: (a) a statistically significant increase in the average number of dead implantations per pregnant female accompanied by significant reductions

in the average number of living embryos, and (b) a significant increase in the number of females with one or more dead implantations.

The sensitivity of the dominant-lethal test is influenced by the stocks of male and female mice used (67), and the yield of dominant-lethal mutations is affected by the strain and age of females mated with treated males (68). There is good evidence that the ability of fertilized eggs to carry out repair of certain chemically-induced premutational lesions present in the fertilizing sperm varies from one strain to another (68).

Induced dominant-lethal effects can be detected at different stages in spermatogenesis and the stage that is affected may vary depending on the structure of the chemical tested. However, a definitive effect in spermatogonial stem cells has not been demonstrated.

The dominant-lethal test can only be used to assess an agent's mutagenic potential in qualitative terms. Furthermore, although dominant-lethal effects are, for all practical purposes, caused by induced chromosome breakage in germ cells, they are not always associated with viable reciprocal exchanges (heritable translocations). For most chemicals studied thus far, there appears to be a close correlation between induction of dominant-lethal mutations and induction of heritable translocations, but there are examples of chemicals that effectively induced dominant-lethal mutations with very few or no heritable translocations (69, 70). A definitive positive response in a dominant-lethal test serves as a strong indication that a test chemical causes genetic damage in male germ cells. However, because the endpoints of dominant-lethal effects are generally subject to considerable variability (from a statistical standpoint) and because of the biological factors described above that affect sensitivity, a negative response is not likely to

be conclusive.

Heritable Translocation Test

The heritable translocation test detects symmetrical reciprocal exchanges of chromosomal material between nonhomologous chromosomes which are transmitted from parents to offspring. Carriers of these exchanges, referred to as translocation heterozygotes, are highly viable and generally cannot be distinguished from normal mice by casual observation. Because heritable translocations are by definition scored among live progeny, they provide a very definitive and unequivocal measure of chromosome-breakage effects. Consequently, they are generally considered to be the most important endpoint of induced chromosome aberrations with respect to genetic risk assessment.

Like dominant-lethal mutations, induction of heritable translocations is stage-dependent. So far, clear-cut inductions of heritable translocations have been observed only from treated meiotic and postmeiotic germ cells of males. Among chemicals studied so far, only alkylating agents have been clearly shown to induce heritable translocations.

The heritable translocation test is carried out in the following sequence: (a) treatment of parental males, (b) mating of treated males with untreated females and production of first-generation progeny and (c) testing of male progeny for translocation heterozygosity. Unlike the dominant-lethal test, it is not practical to use the single dose or the five consecutive days regimen followed by a long-term mating schedule. The practical method of treatment is to subject parental males to long-term exposure at the maximally-tolerated dose. Because heritable translocations are inducible by chemical mutagens only in meiotic and postmeiotic stages, males are exposed continuously, for a minimum of 5 weeks, to allow for effects the chemical may have on

germ-cell maturation which may increase subsequent sensitivity to chemically-induced chromosomal lesions. Because the primary use of the heritable translocation data is in genetic risk assessment, the route of administration should be similar to human situations. Each treated male is caged with two untreated females usually for a period of one week immediately after the end of treatment. All male progeny are weaned and all female progeny are discarded. At sexual maturity, male progeny are tested for translocation heterozygosity.

Screening for translocation heterozygotes may be accomplished by using one or two general procedures. One method consists of testing males initially for sterility and semisterility and subsequent cytological analysis of suspect progeny. The other method bypasses the fertility test and all male progeny are subjected to cytological analysis. The nature of translocations that result in male sterility has been studied exclusively (71, 72, 73). The majority of induced cases of F_1 male sterility involves translocation between autosomes (either distal or proximal). Male sterility can also ensue from translocations between sex-chromosomes and autosomes. Semisterility among translocation heterozygotes is the consequence of the formation of balanced and unbalanced gametes during meiotic segregation (Figure 1). When chromosomes separate by alternate segregation, two normal sperms and two sperms that carry balanced translocations are produced from each spermatocyte. On the other hand, when the chromosomes separate by adjacent I or II segregation, the sperm cells that arise are unbalanced having both duplication and deficiency of chromosome regions. These germ cells with unbalanced chromosomes are capable of fertilization but they lead to early embryonic lethality observed primarily as resorption moles. The presence of multiple chromosome associations, such as, chain and ring configurations in diakinesis-

metaphase I spermatocytes, characterizes translocation heterozygotes.

Observations of these configurations are dependent upon chiasma formation between homologous regions of the chromosomes during meiosis. Meiotic cells for cytological analysis of translocations are routinely prepared using the air-drying technique (74).

The spontaneous occurrence of chromosome breakage-related anomalies in humans is estimated to be 2,400 per million live births (35), most of which are reciprocal and Robertsonian translocations and unbalanced segregation products of translocations. The manifestations of translocations in human carriers are varied ranging from no perceptible effect to mentally-related handicaps (35). For example, approximately 20% of the trisomy 13 disorders (Patau's syndrome) are caused by translocations (75, 76). The exchange usually involves the transfer of material from chromosome 13 to chromosome 14. Although the total contribution that heritable translocations make toward all chromosomal disorders has not been determined adequately, it may be assumed for purposes of estimating human risk, that all newly-occurring translocations are deleterious if not directly to the carriers, to some of their conceptuses. Thus, until accurate human data become available, it may be sufficient to estimate the risk from mouse heritable translocation data as a multiple of the spontaneous rate similar to the indirect method described previously. In cases of prolonged human exposure, the fact that meiotic and postmeiotic stages represent only a fraction of the spermatogenic maturation process becomes less important.

Figure 1. Example of reciprocal translocation between nonhomologous chromosomes in the treated male that is mated with untreated females.

Nonhomologous Chromosomes

A B C D E F G

U V W X Y Z

Translocation
between AB
and UV regions

U V C D E F G

A B W X Y Z

sperm

cross between treated male
containing the reciprocal
translocation and normal
female

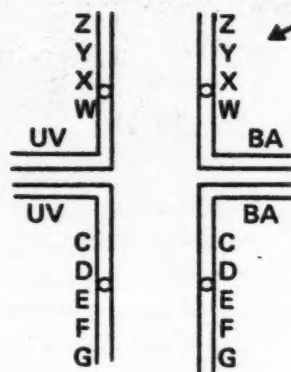
U V C D E F G

A B W X Y Z

F₁ male

A B C D E F G

U V W X Y Z



yields quadrivalent at the
diakinesis stage of meiosis I

alternate segregation

ABCDEFGF, UVWXYZ

2 normal sperm

UVCDEFG, ABWXYZ

2 balanced sperm

adjacent segregation -1

UVWXYZ, ABWXYZ

2 unbalanced sperm

ABCDEFGF, UVCDEFG

2 unbalanced sperm

adjacent segregation -2

ABCDEFGF, ABWXYZ

2 unbalanced sperm

UVCDEFG, UVWXYZ

2 unbalanced sperm

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Sex Chromosome-numerical-anomaly Test

Approximately 0.6% of human newborns have congenital anomalies which result from chromosomal aberrations. These may be unbalanced segregation products of translocations or numerical aberrations (24). Numerical anomalies are known for both autosomes and sex chromosomes. They include Trisomy 21, Down's syndrome [incidence 0.1 to 0.2% live births (77)], trisomy 18, Edward's syndrome [incidence 0.014 to 0.03% live births (78)], trisomy 13, Patau's syndrome [incidence 0.02% live birth (79)], trisomy XXY, Klinefelter's syndrome [incidence (about 0.07%) female live births (80)], and monosomy XO, Turner's syndrome [incidence 0.04% live births (81)]. In general, numerical anomalies of autosomes result in severe clinical manifestations, whereas those of sex chromosomes have a somewhat less debilitating but still severe effect. Chromosomal abnormalities of the numerical type may be brought about by nondisjunction or by chromosomal loss during spermatogenesis or oogenesis which results in aneuploidy (either a missing or extra chromosome) in mature germ cells. When such germ cells combine with those of normal individuals, the resulting offspring could have any of the disorders described above.

A numerical sex chromosome-loss test using mice has been described by Russell (82, 83). Potentially mutagenic agents can be tested in either sex and numerical chromosomal disorders can be scored in the F_1 generation. When males are treated, different stages of spermatogenesis can be sampled by mating at the appropriate time intervals with untreated females. By having genetic markers either on the two X chromosomes of the females or the X chromosome of the male, so that the X's are distinguishable as to parental origin (X^1 and X^2 in Table 3), and by observing the sex and phenotype of the F_1 generation, the incidence of sex chromosomal aneuploidies can be obtained. Table 3 summarizes the possible outcomes.

TABLE 3. SEX CHROMOSOMAL ANEUPLOIDY OUTCOMES IN OFFSPRING OF TREATED MALE MICE (x^2y) DURING MEIOSIS AND MATING WITH FEMALES (x^1x^1)*

Karyotype	Markers Present	Sex	Mutagenic event in Male Parent †
x^10	1	F	NMI, NMII, LMI, LMII
x^1x^2y	1,2	M	NMI
x^1yy	1	M	NMII
$x^1x^2x^2$	1,2	F	NMII
x^1x^2	1,2	F	None
x^1y	1	M	None

*Compiled from data taken from Russell 1976 (82). The superscripts designate gene markers.

†N, nondisjunction; L, chromosomal loss; MI, first meiotic division, MII, second meiotic division.

When females are treated and mated to untreated males, a similar experimental scheme can be used (except that the cross must be of the type $X^1X^2 \times X^3Y$ (all 3 Xs distinguishable, but one of the three may be wild-type)).

The spontaneous frequencies of maternal and paternal sex-chromosome loss are about 5×10^{-4} and 2×10^{-3} , respectively, and that of sex chromosome trisomy is about 1×10^{-4} (82). Large numbers of animals are necessary to obtain dose-response type relationships at the low exposure levels that humans may encounter, and one must therefore work with relatively high experimental doses. If experimental data on an environmental agent are known, then an estimate of human risk can be attempted by extrapolation. For example, if mouse experiments show that a certain exposure to a chemical agent doubles the incidence of sex chromosome loss (XO), then the incidence of Turner's syndrome in humans may be multiplied by the same factor following "equivalent" exposure to the same type of germ cells.

One has to assume that an agent causing a positive response in a numerical sex chromosome study would also cause numerical anomalies (monosomy or trisomy) among autosomes. Therefore, humans exposed to the chemical agent would be at risk to other diseases caused by autosomal numerical aberrations. Only four chemicals (triethylenemelamine, methyl methanesulfonate, isopropylmethanesulfonate, and hycanthone) and ionizing radiation have been tested in the mouse numerical-sex chromosome anomaly system (82). All of these agents are also known to cause dominant lethals. The sex chromosome system is the only genetic test presently available to assess the in vivo mutagenic activity of chemicals which cause nondisjunction. Mechanisms of nondisjunction [e.g., interfering with the mitotic/meiotic spindle (tubulin polymerization) or crossover depression] may not involve a direct interaction with DNA.

DISCUSSION

In 1977 a report was published on the "Approaches to Determining the Mutagenic Properties of Chemicals: Risk to Future Generations" (84) by a working group of the subcommittee on environmental mutagenesis for the Department of Health, Education, and Welfare. This was the first report which dealt with test procedures and approaches to determine if chemicals in the environment possess mutagenic activity as well as whether these test systems are relevant in the assessment of human genetic risk to future generations.

In 1979 the first Banbury report was published on biological risk assessments of chemical mutagens (85). The report consists of a series of papers that consider current concepts and approaches for estimating human mutagenic risks. Many of these concepts have been applied to the in vivo test methods discussed in this chapter.

In 1980 Russell and Matter (86) evaluated five whole-mammal mutagenicity test methods and discussed the advantages and limitations for assessing mutagenic activity of chemical agents. This latest review provides some current thinking on how to use various mammalian mutagenic test systems for estimating human risks. It is quite apparent from the many assumptions that mutagenic risk assessment is at the developing stages and should be conducted cautiously. However, because of the many environmental agents that have been identified as being potentially mutagenic to humans and recent public recognition of the potential problem, regulatory agencies must make judgments based on current scientific knowledge to protect public health.

In 1980 the U.S. Environmental Protection Agency proposed mutagenicity risk assessment guidelines (25) in order to provide a framework under its various statutes to analyze mutagenicity data. The proposed guidelines suggest the use of expert consultants and advisors in making mutagenicity

risk assessments for environmental agents and identify the need to revise the guidelines as more knowledge becomes available. Hopefully, both government and the private sector will continue to develop and validate mutagenic risk methodologies so that such evaluations can be used for more accurate assessments in the future.

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